

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 16, line 5, and replace it with the following paragraph:

Figure 1a: Vector map of construct pMorphX7-hag2-LH (The 6xHis tag shown is SEQ ID NO: 11).

Please delete the paragraph on page 16, line 7, and replace it with the following paragraph:

Figure 1b: Vector sequence of pMorphX7-hag2-LH (SEQ ID NO: 35).

Please delete the paragraph on page 16, line 9, and replace it with the following paragraph:

Figure 2: Vector sequence of pTFT74-N1-hag-HIPM (SEQ ID NO: 36).

Please delete the paragraph on page 16, line 11, and replace it with the following paragraph:

Figure 3: Vector sequence of pQE60-MacI (SEQ ID NO: 37).

Please delete the paragraph on page 17, lines 3-4, and replace it with the following paragraph:

Figure 6b: Sequence of expression cassette for full length pIII with an N-terminal cysteine residue (C-gIII) (SEQ ID NO: 38).

Please delete the paragraph on page 17, lines 6-7, and replace it with the following paragraph:

Figure 6c: Sequence of expression cassette for truncated pIII with an N-terminal cysteine residue (C-gIIICT) (SEQ ID NO: 39).

Please delete the paragraph on page 17, line 9, and replace it with the following paragraph:

Figure 7a: Vector map of construct pMorph18-C-gIII- hag2-LHC (The 6xHis tag shown is SEQ ID NO: 11).

Please delete the paragraph on page 17, line 11, and replace it with the following paragraph:

Figure 7b: Vector sequence of pMorph18-C-gIII-hag2-LHC (SEQ ID NO: 40).

Please delete the paragraph on page 19, line 33, and replace it with the following paragraph:

Figure 16a: Vector map of construct pMorphX10-Fab-MacI5-VL-LHC-VH-FS
(The 6xHis tag shown is SEQ ID NO: 11).

Please delete the paragraph on page 20, line 1, and replace it with the following paragraph:

Figure 16b: Complete vector sequence of pMorphX10-Fab-MacI5-VL-LHC-VH-FS
(SEQ ID NO: 41).

Please delete the paragraph on page 23, lines 12-24, and replace it with the following paragraph:

All vectors used are derivatives of the high copy phagemid pMorphX7-LH (Figure 1a + b), a derivative of the pCAL vector series (WO 97/08320; Knappik et al., 2000). The expression cassette comprises the phoA signal sequence, a minimal binding site for the monoclonal antibody (mab) anti-FLAG M1 (Sigma #F-3040) (Knappik and Plückthun, 1994), a single chain fragment (scFv), a short linker (PGGSG, SEQ ID NO: 10) and a 6x histidine tag (6His SEQ ID NO: 11); Hochuli et al., 1988) (Figure 1a). pMorphX7-LCH and pMorphX7-LHC have been generated by inserting oligonucleotide cassettes coding for Cys-6His SEQ ID NO: 12 and 6His-Cys SEQ ID NO: 13, respectively, between the unique *Ascl* and *HindIII* sites of pMorphX7-LH (Figure 1a, Table 1). All vectors express soluble scFv not genetically fused to any phage coat protein. The conventional phage display vector pMorph13 which is based on the pCAL4 vector described in WO 97/08320 and expresses a fusion of an scFv to the C-terminal part of phage protein pIII was used as positive control. The scFvs have been

exchanged between the respective vectors via the unique *Xba*I and *Eco*RI sites (c.f. Figure 1a).

Please delete the paragraph on page 23, line 29, to page 24, line 19, and replace it with the following paragraph:

All scFvs derive from a human combinatorial antibody library (HuCAL; WO 97/08320; Knappik et al., 2000). The HuCAL VH and VL consensus genes (described in WO 97/08320), and the CDR3 sequences of the scFvs are given in Table 2. Clone hag2 was selected against a peptide from influenza virus hemagglutinine (aa 99-110 from hemagglutinine plus additional flanking aa (shown in *italics*, CAGPYDVPDYASLRSHH, SEQ ID NO: 14), and clone Macl-5 against a fragment (Macl) of human CR-3 alpha chain (SWISS-PROT entry P11215, aa 149 – 353 of human CR-3 alpha fused to a C-terminal sequence containing a 6x histidine tag (SEQ ID NO: 11)). The corresponding antigens for ELISA and doped library experiments were obtained as follows. The hag2 specific antigen N1-hag was produced using expression vector pTFT74-N1-hag-HIPM, a derivative of vector pTFT74 (Freund et al., 1993) (Fig. 2). N1-hag comprises aa 1-82 of mature gene III protein of phage M13 containing an additional methionine residue at the N-terminus (N1) fused to the amino acid sequence PYDVPDYASLRSHHHHHH (SEQ ID NO: 1) (hag) comprising aa 99-110 from influenza virus hemagglutinine and a 6x histidine tag (SEQ ID NO: 11) (in *italics*). Expression, purification and refolding of N1-hag was done as described (Krebber, 1996; Krebber et al., 1997). As antigen for Macl-5, a purified fragment (Macl) of human CR-3 alpha chain (SWISS-PROT entry P11215) fused to a C-terminal 6x histidine tag (SEQ ID NO: 11) was used. In detail, the expression cassette encodes an N-terminal methionine, amino acids 149 – 353 of human CR-3 alpha and amino acids IEGRRHHHHH (SEQ ID NO: 2). This cassette is flanked by unique restriction sites *Bsp*HI and *Hind*III and can e.g. be introduced into the unique *Nco*I and *Hind*III sites of pQE-60 (QIAGEN GmbH, Hilden, Germany), yielding expression vector pQE60-Macl (Fig. 3). Expression and purification was performed using standard methods (The QIAexpressionist™ 3rd edition: A handbook for high-level expression and purification of 6xHis-tagged (SEQ ID

NO: 11) proteins (July 1998). QIAGEN GmbH, Hilden, Germany). Bovine serum albumin (BSA, Sigma #A7906) was used as negative control antigen.

Please delete the paragraph on page 27, lines 7-20, and replace it with the following paragraph:

Phage coat protein expression cassettes for the two-vector system were constructed as follows: Two different expression cassettes flanked by unique *NheI* and *HindIII* restriction sites at the ends were made positioning an unpaired cysteine residue at the exposed N-terminus of the N1-domain of full length mature pIII (C-gIII) or at the N-terminus of the pIIICT domain of the truncated protein (amino acids 216 to 406 of protein pIII; C-gIIICT) (Fig. 6b + c)). Both expression cassettes are under the control of the lac promoter/operator region and comprise the signal sequence ompA, amino acids DYCDIEF (SEQ ID NO: 3) and the pIII or pIIICT ORF (complete amino acid sequences are given in Table 3). Plasmids expressing the modified pIII proteins were obtained by inserting these *NheI-HindIII* cassettes into plasmid pBR322 and pUC19 via the unique *NheI* and *HindIII* or *XbaI* and *HindIII* sites, respectively. As an example, the vector map of pBR-C-gIII is depicted in Figure 6a. The resulting plasmids, pBR-C-gIII, pBR-C-gIIICT, pUC-C-gIII and pUC-C-gIIICT, were co-transformed with pMorphX7-LHC phagemids expressing the modified scFv (Example 1) into *E.coli* TG1 selecting for both antibiotic markers.

Please delete the paragraph on page 27, line 22, to page 28, line 7, and replace it with the following paragraph:

In the one-vector system both the modified phage coat proteins as well as the modified scFv were expressed from a dicistronic phagemid under control of the lac promoter/operator region. The first expression cassette comprises the signal sequence ompA, amino acids DYCDIEF (SEQ ID NO: 3) and the ORF for the respective phage coat protein or part thereof. The unpaired cysteine residue was linked to the exposed N-terminus of the N1-domain of full length mature pIII (C-gIII), to the N-terminus of the truncated protein III (amino acids 216 to 406 of protein pIII; C-gIIICT) and to the N-

terminus of protein IX (C-gIX), respectively (amino acid sequences are given in Table 4). The second expression cassette comprises the *phoA* signal sequence, the ORF of the respective scFv, a short linker (PGGSG, SEQ ID NO: 10), a 6x histidine tag (6His (SEQ ID NO: 11); Hochuli et al., 1988) and the single cysteine residue (see pMorphX7-LHC, Table 1). The complete vector sequence of pMorph18-C-gIII-hag2-LHC coding for modified full length pIII as well as modified scFv hag2 and the respective vector map are given in Figure 7a+b. The different phage coat proteins can be exchanged via *EcoRI* and *StuI* in a three fragment cloning procedure due to a second *EcoRI* site at the 3' end of the scFvs. The different engineered scFvs can be cloned via the unique *MfeI* and *HindIII* sites. A derivative of this vector, pMorph20-C-gIII-hag2-LHC, contains a unique *EcoRI* site at the 3' end of the scFv while the second site (between the *ompA* signal sequence and the gIII ORF) was deleted via silent PCR mutagenesis. This construct allows the cloning of scFvs or scFv pools via the unique *SphI* and *EcoRI* sites.

Please delete the paragraph on page 32, lines 13-24, and replace it with the following paragraph:

Pannings were performed against the following antigens: (i) ICAM1 comprising the extracellular part of mature ICAM1 (amino acids 1-454) plus amino acids CGRDYKDDDKHHHHHH (SEQ ID NO: 4) containing the M2-Flag and the 6x histidine tag (SEQ ID NO: 11). (ii) N1-Macl comprising aa 1-82 of mature gene III protein of phage M13 containing an additional methionine residue at the N-terminus plus a short linker at the C-terminus (N1), fused to a polypeptide containing amino acids 149-353 of human CR-3 alpha chain (SWISS-PROT entry P11215) plus the C-terminal sequence IEGRHHHHHH (SEQ ID NO: 2) which includes the 6x histidine tag (SEQ ID NO: 11); and (iii) N1-Np50 comprising N1 fused to a polypeptide containing amino acids 2-366 of human NFkB p50 plus amino acids EFSHHHHHH (SEQ ID NO: 5) which include the 6x histidine tag (SEQ ID NO: 11). Expression vectors for N1-Macl and N1-Np50 are based on vector pTFT74 (Freund et al., 1993) (complete vector sequence of pTFT74-

N1-hag-HIPM given in Fig. 2). Expression, purification and refolding was done as described (Krebber, 1996; Krebber et al., 1997).

Please delete the paragraph on page 33, line 23, to page 34, line 11, and replace it with the following paragraph:

After each round of panning the number of antigen specific phages was determined in an ELISA. N1-MacI, N1-Np50 and ICAM-Strep (comprising amino acids 1-455 of mature ICAM1 plus *SAWSHPQFEK* (SEQ ID NO: 6) containing the Strep-tag II) were used as antigens, respectively. To ensure high level expression the selected scFvs were subcloned into expression vector pMorphX7-FS (Table 1). Subcloning was done in two steps. First the scFv fragments were isolated from pMorph20-C-gIII-scFv-LHC via *Afl* and *Eco*RI, then the fragments were re-digested with *Sph*I and cloned into the *Eco*RI/*Sph*I digested pMorphX7-FS vector. This procedure ensured that only scFvs from vector pMorph20-C-gIII-scFv-LHC were subcloned and excluded any contamination with scFvs from a conventional display or expression vector. Expression of the scFvs and their testing in ELISA against the respective antigens was done according to standard procedures. Clones which showed a signal of at least 3x above background in ELISA were considered positive. The results are summarised in Table 5. To prove that the selected scFvs bind strongly and specifically to their respective antigen several positive clones after 2 rounds of cys-display panning were selected and re-tested in quadruplicates in a specificity ELISA on six different antigens (Figures 14 & 15). Enrichment of antigen-specific binders could clearly be demonstrated. Already after two rounds of cys-display panning of the pre-selected pools against N1-MacI, N1-Np50 and ICAM1 between 80 % and 97 % of the tested clones were positive in ELISA. The affinity of some of the selected scFvs was determined in Biacore and Kd values in the range of 1 nM to 2.2 μ M were determined. These results are similar to the enrichment factors and affinities obtained in a conventional panning of the respective pools performed in parallel. Some of the scFvs were selected independently via cys-display as well as conventional panning.

Please delete the paragraph on page 35, line 27, to page 36, line 4, and replace it with the following paragraph:

Two different plasmids were used for expression of full length pIII. Plasmid pBR-C-gIII was already described above. The respective expression cassette comprises the signal sequence ompA, amino acids DYCDIEF (SEQ ID NO: 3) and the pIII ORF under control of the lactose promotor/ operator region (Table 3, Figure 6). Alternatively, plasmid pBAD-SS-C-gIII was used. Here the respective expression cassette comprises the signal sequence of pIII, amino acids TMACDIEF (SEQ ID NO: 7) and the pIII ORF under control of the arabinose promotor/operator region (Table 3). For construction of pBAD-SS-C-gIII the fragment coding for the engineered cysteine plus pIII was amplified from pUC-C-gIII via PCR introducing the restriction sites *NcoI* and *HindIII* and cloned into the commercially available vector pBAD/gIII A (Invitrogen). The plasmids pBR-C-gIII or pBAD-SS-C-gIII were co-transformed with the respective pMorphX10-Fab phagemids expressing the modified Fab into *E.coli* TG1 selecting for both antibiotic markers.

Please delete the paragraph on page 36, lines 8-26, and replace it with the following paragraph:

Three different Fabs all deriving from a human combinatorial antibody library (HuCAL; WO 97/08320; Knappik et al., 2000) were used for evaluation of Fab display on engineered phage. The HuCAL VH and VL consensus genes (described in WO 97/08320), and the CDR3 sequences of the Fabs are given in Table 2. Fab MacI-5 is derived from the scFv MacI-5 described above and was converted into the Fab format (complete vector map of pMorphX10-Fab-MacI5-VL-LHC-VH-FS is given in Figure 16a). Fabs MacI-A8 and ICAM1-C8 were isolated directly from one of the HuCAL-Fab libraries. Clone MacI-A8 was selected against antigen MacI-Strep, which comprises an N-terminal methionine, amino acids 149-353 of human CR-3 alpha chain (SWISS-PROT entry P11215) and amino acids SAWSHPQFEK (SEQ ID NO: 6) which

include the Strep-tag II (Schmidt et al., 1996). Expression and purification were done according to Schmidt & Skerra (1994). N1-MacI was used as corresponding antigen for ELISAs. N1-MacI is described above, and comprises an N-terminal methionine, amino acids 1-82 of mature gene III protein of phage M13 plus a short linker (N1), amino acids 149-353 of human CR-3 alpha chain (SWISS-PROT entry P11215) and amino acids IEGRHHHHHH (SEQ ID NO: 2) which include the 6x histidine tag (SEQ ID NO: 11). Clone ICAM1-C8 was selected against antigen ICAM1 described above, which comprises the extracellular part of mature ICAM1 (amino acids 1-454) plus amino acids CGRDYKDDDKHHHHHH (SEQ ID NO: 4) containing the M2-Flag and the 6x histidine tags (SEQ ID NO: 11). The same antigen was used for ELISA assays as well as in the doped library experiment.

Please delete Table 1, and replace it with the following Table:

Table 1: Amino acid sequence of ORF modules between the *EcoRI* and *HindIII* sites of vectors pMorphX7-hag2-FS, pMorphX7-hag2-LH, pMorphX7-hag2-LCH and pMorphX7-hag2-LHC

Construct	<i>EcoRI</i>	Module 1	<i>AseI</i>	Module 2	<i>HindIII</i>
pMorphX7-FS	EF	DYKDDDDK (SEQ ID NO: 8)	GAP	WSHPQFEK-stop (SEQ ID NO: 9)	stop
pMorphX7-LH	EF	PGGSG (SEQ ID NO: 10)	GAP	HHHHHH-stop (SEQ ID NO: 11)	stop
pMorphX7-LCH	EF	PGGSG (SEQ ID NO: 10)	GAP	CHHHHH-stop (SEQ ID NO: 12)	stop
pMorphX7-LHC	EF	PGGSG (SEQ ID NO: 10)	GAP	HHHHHC-stop (SEQ ID NO: 13)	stop

Please delete Table 2, and replace it with the following Table:

Table 2: Amino acid sequence of HuCAL scFvs and HuCAL Fabs*

scFv	antigen	VH	H CDR3	VL	L CDR3
hag2	peptide of influenza virus hemagglutinine (CAGPYDVPDYASLRSHH) (SEQ ID NO: 14)	VH3	RSGAYDY (SEQ ID NO: 15)	Vκ4	QQYSSFPL (SEQ ID NO: 16)
AB1.1	12 amino acid peptide	VH3	10 amino acid residues	Vλ1	9 amino acid residues
MacI-5	fragment of human CR-3 alpha chain	VH2	FDPFFDSFFDY (SEQ ID NO: 17)	Vλ1	QSYDQNALVE (SEQ ID NO: 18)
MacI-A8	fragment of human CR-3 alpha chain	VH3	HGYRKYYTDMFDV (SEQ ID NO: 19)	Vκ1	HQVYSTSP (SEQ ID NO: 20)
ICAM1-C8	human ICAM1	VH2	FPYTYHGFMND (SEQ ID NO: 21)	Vλ3	QSYDSGNL (SEQ ID NO: 22)

* details are given in the Examples

Please delete Table 3, and replace it with the following Table:

Table 3: Amino acid sequence of engineered phage coat proteins of vector pBR-C-gIII and derivatives

Construct	Signal Sequence		EcoRV EcoRI	sequence	HindIII	SEQ ID NO:
pUC-C-gIII pBR-C-gIII	MKKTAlAlAVAL AGFATVAQA (ompA)	DYC	DI EF	AETVESCLAKPHTENSFTNVVK DDKTLDRYANYEGCLWNATGV VVCTGDETCQCYGTWVPIGLAIE NEGGGSEGGGSEGGGSEGGGK PPEYGDTPIPGYTYINPLDGTYP GTEQNPANPNPSLEESQPLNTFM FQNNRFRNRQGALT VYTGTVTQ GTD PVKTY YQYTPVSSKAMYDA YWNGKFRDCAFHSGFNEDPFVC EYQGQSSDLPQPPVNAAGGSGG GSGGGSEGGGSEGGGSEGGGSE	stop	<u>23</u>

				GGGSGGGSGSGDFDYEKMANA NKGAMTENADENALQSDAKGKL DSVATDYGAIDGFIGDVSGLAN GNGATGDFAGSNSQMAQVGDG DNSPLMNNFRQYLPSLPQSVCECR PYVFGAGKPYEFSIDCDKINLFRG VFAFLLYVATFMYVFSTFANILR NKES		
pUC-C- gIICT pBR-C- gIICT	MKKTAAIAVAL AGFATVAQA (ompA)	DYC	DI EF	NAGGGSGGGSGGGSEGGGSEGG GGSEGGGSEGGGSGGGSGSGD FDYEKMANANKGAMTENADEN ALQSDAKGKLDSVATDYGAID GFIGDVSGLANGNGATGDFAGS NSQMAQVGDGDN SPLMNNFRQ YLPSLPQSVCECRPFVFGAGKPYE FSIDCDKINLFRGVFAFLLYVATF MYVFSTFANILRNKES	stop	<u>24</u>
pBAD-SS-C- gIII	MKKLLFAIPLVVP FYSHS (gIII)	TMAC <i>NcoI</i> (<i>Styl</i>) / <i>SphI</i>	DI EF	AETVESCLAKPHTENSFTNVWK DDKTLDRYANYEGCLWNATGV VVCTGDETQCYGTWVPIGLAIP NEGGSSEGGGSEGGGSEGGGK PPEYGDTPIPGYTYINPLDGTYP GTEQNPANPNPSLEESQPLNTFM FQNNRFRNRQGALTIVYTGTVTQ GTDVPKTYQYTPVSSKAMYDA YWNGKFRDCAFHSGFNEDPPVC EYQGQSSDLPPPVNAGGGSGG GSGGGSEGGGSEGGGSEGGGSE GGGSGGGSGSGDFDYEKMANA NKGAMTENADENALQSDAKGKL DSVATDYGAIDGFIGDVSGLAN GNGATGDFAGSNSQMAQVGDG DNSPLMNNFRQYLPSLPQSVCECR PYVFGAGKPYEFSIDCDKINLFRG VFAFLLYVATFMYVFSTFANILR NKES	stop	<u>25</u>

The engineered Cys is written in bold

Sequence of wild type phage coat proteins is underlined

Please delete Table 4, and replace it with the following Table:

Table 4: Amino acid sequence of engineered phage coat proteins of vector pMorph18-C-gIII-scFv-LHC and derivatives

Construct	OmpA Signal Sequence		EcoRV EcoRI	sequence	Stop	SEQ ID NO:
pMorph18-C-gIII-scFv-LHC	MKKT AIAIV ALAGFATVAQ A	DYC	DI EF	AETVESCLAKPHNTENSFTNV WKDDKTLDRYANYEGCLWN ATGVVVCTGDETCYGTWV PIGLAIPENEGGSGEGGSEG GGSEGGGTPPEYGDTPIG YTYINPLDGYPPGTEQNAN PNPSLEESQPLNTFMFQNNRF RNRQGALTVYTGTVTQGT PVKTYQYTPVSSKAMYDA YWNGKFRDCAFHSGFNEDPF VCEYQGQSSDLPQPPVNAG GGSGGGSGGGSEGGGSEGG GSEGGGSEGGSGGGSGSG DFDYKMANANKGAMTENA DENALQSDAKGKLDVSATD YGAAIDGFIGDVSGLANGNG ATGDFAGSNSQMAQVGDG DNSPLMNNFRQYLPSPQSV ECRPYVFGAGKPYEFSIDCK INLFRGVFAFLLYVATFMYVF STFANILRNKES	stop	<u>26</u>
pMorph18-C-gIIICT-scFv-LHC	MKKT AIAIV ALAGFATVAQ A	DYC	DI EF	NAGGGSGGGSGGGSEGGGS EGGGSEGGGSEGGSGGGSG SGDFDYKMANANKGAMTE NADENALQSDAKGKLDVSAT DYGAAIDGFIGDVSGLANGNG ATGDFAGSNSQMAQVGDGD NSPLMNNFRQYLPSPQSV RPFVFGAGKPYEFSIDCKINL FRGVFAFLLYVATFMYVFSTF ANILRNKES	stop	<u>27</u>

pMorph18-C- glX-scFv- LHC	MKKTAAIAV ALAGFATVAQ A	DYC	DI EF	GGGGSMSVLVYSFASVFLGW CLRSGITYFTRLMETSS	stop	28
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The engineered Cys is written in bold

Sequence of wild type phage coat proteins is underlined

Please delete Table 6, and replace it with the following Table:

Table 6: Amino acid sequence of modules of engineered Fab fragment

Construct	Module at the light chain		Module at the heavy chain	
	elements	amino acids	elements	amino acids
pMorphX10-Fab- VL-LHC-VH-FS	linker- histidine tag- cysteine	SPGGSG-GAP- HHHHHH- C-stop (SEQ ID NO: 29)	linker Flag tag-linker Strep-tag II	EF- DYKDDDDK-GAP- WSHPQFEK-stop (SEQ ID NO: 30)
pMorphX10-Fab- VL-LHC-VH-MS	linker- histidine tag- cysteine	SPGGSG-GAP- HHHHHH- C-stop (SEQ ID NO: 29)	linker- myc tag-linker- Strep-tag II	EF- EQKLISEEDLN-GAP- WSHPQFEK-stop (SEQ ID NO: 31)
pMorphX10-Fab- VL-C-VH-FS	cysteine	deletion of A- C-stop (κ -chains) CS-stop (λ -chains)	linker- Flag tag-linker- Strep-tag II	EF- DYKDDDDK-GAP- WSHPQFEK-stop (SEQ ID NO: 30)
pMorphX10-Fab- VL-C-VH-MS	cysteine	deletion of A- C-stop (κ -chains) CS-stop (λ -chains)	linker- myc tag-linker- Strep-tag II	EF- EQKLISEEDLN-GAP- WSHPQFEK-stop (SEQ ID NO: 31)
pMorphX10-Fab- VL-VH-LHC	-		linker- histidine tag- cysteine	EF-PGGSG-GAP- HHHHHH- C-stop (SEQ ID NO: 32)
pMorphX10-Fab- VL-VH-CFS	-		cysteine-linker- Flag tag-linker- Strep-tag II	C-EF- DYKDDDDK-GAP WSHPQFEK-stop (SEQ ID NO: 33)
pMorphX10-Fab- VL-VH-CMS	-		cysteine-linker- myc tag-linker- Strep-tag II	C-EF- EQKLISEEDLN-GAP- WSHPQFEK-stop (SEQ ID NO: 34)

The engineered cysteine is written in bold